

Formulations

All powdered media are produced without sodium bicarbonate to increase their stability and shelf life. L-glutamine is included in all media where the author's original formulation requires. As with liquid media, individual components of production lots may be adjusted by the substitution of hydrated or anhydrous form, and free base or hydrochloride form to compensate for availability of various raw materials. The appropriate weight per litre is given on all package labels.

Storage/Handling

Powdered media are extremely hygroscopic and must be stored in airtight, sealed containers. If exposed to air, caking and chemical degradation will occur in these products. Refrigeration at 4°C with desiccation prolongs the storage period. We strongly discourage the practice of opening and closing powdered media containers in order to weigh out smaller aliquots than the stated package volumes.

Concentrates

We do not recommend preparing concentrated media from our powdered products. Attempts to prepare 5 times (5X) and 10 times (10X) concentrated liquid media from powder will often result in the formation of salt complexes with resulting precipitation. The solubility coefficients for many free base amino acids may be exceeded and, as a result, these components will not completely dissolve.

Preparation of Single Strength Solution from Dry Powdered Media**A. (Cat. No. 11700)**

(Cat. No. 21800)

(Cat. No. 21400)

(Preparation of Single Strength Autoclavable Medium)

1. Dissolve contents of 1L package in 950 ml deionised-distilled water.
2. Adjust pH to 4.1-4.2 with 1N HCl.
3. Dispense 95 ml into 100 ml bottles.
4. Autoclave for 15 minutes at 121°C. Exhaust on slow cycle.
5. Allow to cool at room temperature.
6. Add 3 ml sterile 7.5% NaHCO₃ solution to each bottle. Adjust to final pH 7.2-7.4 with sterile 1N NaOH if necessary.
7. Aseptically add L-glutamine, serum, antibiotics and other supplements as desired.

B. (Cat. No. 41300)

(Preparation of Single Strength Leibovitz's L-15)

1. Measure out 5% less water (deionised-distilled) than desired total volume of medium, using a mixing container that is as close to the final volume as possible.
2. Add powdered medium to water at room temperature (15°C-20°C) with gentle stirring. (Do not heat water). Rinse out inside of package to remove all traces of powder.
3. Stir until dissolved. (Do not over mix).
4. If necessary, adjust pH to 7.6 with either 1N NaOH or 1N HCl. (Slowly, with stirring).
5. Dilute to desired volume with water. Keep container closed until medium is filtered.
6. Sterilise immediately by membrane filtration. (Air pressure system recommended).

C. (Cat. No. 42200)

Preparation of Single Strength IMDM

1. Measure out 5% less water (deionised-distilled) than desired total volume of medium, using a mixing container that is as close to the final volume as possible.

2. Add powder medium to water at room temperature (15°C- 20°C) with gentle stirring. (Do not heat water).
3. Rinse out inside of package to remove all traces of powder.
4. Add 3.024 grams of NaHCO₃ per litre of medium.
5. Dilute to desired volume with water. Stir until dissolved.
6. Do Not Adjust pH. Keep container closed until medium is filtered
7. Sterilise immediately by membrane filtration (Positive pressure system recommended).

References

Greene, A. E. and Coriell, L. L., Virus Replication in Premixed Powdered Tissue Culture Media *American Journal of Epidemiology*, Vol. 83, No. 1, 1965.
 Green, A. E., Silver, R. K., Krug, M. D. and Coriell, L. L., A Premixed Powder for Preparation of Tissue Culture Media, *Proceedings of the Society for Experimental Biology and Medicine*, V. 111, 122-128, 1965.
 Haylick, L., Jacobs, P. and Perkins, A., Procedure for the Standardisation of Tissue Culture Media, *Nature*, Vol. 1, pp. 146-147, October 10, 1964.
 Sandford, K. K., Hoemann, R. E., Bryant, J. C., Shannon, J. R. and Evans, V. J., Premixed Powders for Protein-Free Cell Culture Media: A Quantitative Evaluation, *Journal of the National Cancer Institute*, Vol. 40, No. 5, May 1968.
 Young, P. B., Sharon, W. S. and Long, R. B., Preparation and Use of Dry Powder Tissue Culture Media, *Annals of the New York Academy of Sciences*, Vol. 139, Article 1, pp. 108-110, October 7, 1966.

Preparation of Single Strength Solution from Dry Powdered Media, Recommended Bicarbonate Levels/L Media

Cat. No.	Preparation Method	NaHCO ₃ g/L	m/L 7.5 % Solution	Recommended working pH
41300	B	0	0	7.3-7.6
21200	D	0.35	4.7	7.1-7.4
41200	D	0.35	4.7	7.0-7.4
41600	D	0.35	4.7	7.0-7.4
31200	D	0.35	4.7	7.0-7.4
11623	D	0.35	4.7	7.0-7.4
10012	D	0.35	4.7	6.0-6.2
11012	D	0.35	4.7	7.0-7.4
13018	B	0.85	11.35	7.0-7.4
20011	D	0.85	11.35	7.0-7.4
21011	D	0.85	11.35	7.0-7.4
13016	D	0.85	11.35	7.0-7.4
71100	D	1.125	15	7.1-7.4
42400	D	1.125	15	7.1-7.4
21700	D	1.176	15.7	7.6-7.9
81200	D	1.2	16	7.0-7.4
22300	D	2	26.7	7.0-7.4
51800	B	2	26.7	7.0-7.4
61100	D	2.2	29.3	7.0-7.4
11400	D	2.2	29.3	7.0-7.4
41500	D	2.2	29.3	7.0-7.4
41100	D	2.2	29.3	7.0-7.4
31300	D	2.2	29.3	7.0-7.4
21600	D	2.2	29.3	6.6-7.1
11624	D	2.2	29.3	6.6-7.1
11900	D	2.2	29.3	7.0-7.4
12000	D	2.2	29.3	7.0-7.4
31100	D	2.2	29.3	7.0-7.4
11700	A	2.2	3ml/95ml after autoclaving	7.0-7.4
21800	A	2.2	3ml/95ml after autoclaving	7.0-7.4
21400	A	2.2	3ml/95ml after autoclaving	7.0-7.4
51400	D	2.24	29.9	7.1-7.4
22100	D	2.75	36.6	6.7-7.1
32200	D	2.75	36.6	6.7-7.1
42200	C	3.024	40.4	7.0-7.4
31600	D	3.7	49.3	7.0-7.4
52100	D	3.7	49.3	7.0-7.4

Note: If HEPES is chosen as a buffer the bicarbonate level should not exceed 0.85 g/L. Bicarbonate levels higher than this will result in problems with osmolality unless this is compensated by a reduction in the salt concentration.

Synthetic Culture Media

Iscove's Modified Dulbecco's Medium (IMDM)^{1,2,3}

Cat No.	21980 1X Liquid	42200 Powder	41980 1X Liquid	31980 1X Liquid
Component	mg/L	mg/L	mg/L	mg/L
INORGANIC SALTS:				
CaCl ₂ (anhyd.)	-	165.00	-	-
CaCl ₂ • 2H ₂ O	219.00	-	219.00	219.00
KCl	330.00	330.00	330.00	330.00
KNO ₃	0.076	0.076	0.076	0.076
MgSO ₄ (anhyd.)	200.00	97.67	200.00	200.00
NaCl	4505.00	4500.00	4505.00	4505.00
NaHCO ₃	3024.00	-	3024.00	3024.00
NaH ₂ PO ₄ • H ₂ O ^c	-	125.00	-	-
NaH ₂ PO ₄ • 2H ₂ O	141.00	-	141.00	141.00
Na ₂ SeO ₃ • 5H ₂ O	0.017	0.017	0.017	0.017
OTHER COMPONENTS:				
D-Glucose	4500.00	4500.00	4500.00	4500.00
Phenol Red	15.00	15.00	15.00	15.00
HEPES	5958.00	5958.00	5958.00	5958.00
Sodium Pyruvate	110.00	110.00	110.00	110.00
AMINO ACIDS:				
L-Alanine	25.00	25.00	25.00	25.00
L-Asparagine • H ₂ O	28.40	-	28.40	28.40
L-Asparagine (freebase)	-	25.00	-	-
L-Arginine • HCl	84.00	84.00	84.00	84.00
L-Aspartic Acid	30.00	30.00	30.00	30.00
L-Cystine • 2HCl	-	91.24	-	-
L-Cystine	70.00	-	70.00	70.00
L-Glutamic Acid	75.00	75.00	75.00	75.00
L-Glutamine	584.00	584.00	-	-
L-Alanyl-L-Glutamine	-	-	-	868.00
L-Glycyl-L-Glutamine	-	-	812.00	-
Glycine	30.00	30.00	30.00	30.00
L-Histidine HCl • H ₂ O	42.00	42.00	42.00	42.00
L-Isoleucine	105.00	105.00	105.00	105.00
L-Leucine	105.00	105.00	105.00	105.00
L-Lysine HCl	146.00	146.00	146.00	146.00
L-Methionine	30.00	30.00	30.00	30.00
L-Phenylalanine	66.00	66.00	66.00	66.00
L-Proline	40.00	40.00	40.00	40.00
L-Serine	42.00	42.00	42.00	42.00
L-Threonine	95.00	95.00	95.00	95.00
L-Tryptophan	16.00	16.00	16.00	16.00
L-Tyrosine (disodium salt)	104.20	104.20	104.20	104.20
L-Valine	94.00	94.00	94.00	94.00
VITAMINS:				
Biotin	0.013	0.013	0.013	0.013
D-Ca Pantothenate	4.00	4.00	4.00	4.00
Choline Chloride	4.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	4.00
i-Inositol	7.20	7.20	7.20	7.20
Nicotinamide	4.00	4.00	4.00	4.00
Pyridoxal HCl	4.00	4.00	4.00	4.00
Riboflavin	0.40	0.40	0.40	0.40
Thiamine HCl	4.00	4.00	4.00	4.00
Vitamin B ₁₂	0.013	0.013	0.013	0.013

1. Dulbecco, R. and Freeman, G. (1959) *Virology* 8, 398. Smith, J. D., Freeman, G., Vogt, M. and Dulbecco, R. (1960) *Virology* 12, 165. Tissue Culture Standards Committee, *In Vitro* 6:2, 93.

2. Iscove, N. N. and Melchers, F. J. *Experimental Medicine* 147, 923.

3. Iscove, N. N. personal communication.

c. Values shown are in conformance with the Tissue Culture Standards committee, *In Vitro* (1970) 9:6

Lactalbumin Hydrolysate (ELH)

Cat No.	31250 1X Liquid	11624 Powder mg/L
Component	mg/L	mg/L
INORGANIC SALTS:		
CaCl ₂ (anhyd.)	-	200.00
CaCl ₂ • 2H ₂ O	264.00	-
KCl	400.00	400.00
MgSO ₄ (anhyd.)	-	97.70
MgSO ₄ • 7H ₂ O	200.00	-
NaCl	6800.00	6800.00
NaHCO ₃	2200.00	-
NaH ₂ PO ₄ • H ₂ O	-	140.00
NaH ₂ PO ₄ • 2H ₂ O	158.00	-
OTHER COMPONENTS:		
D-Glucose	1000.00	1000.00
Lactalbumin Hydrolysate	6500.00	5000.00
Phenol Red	10.00	10.00

Lactalbumin Hydrolysate (HLH)

Cat No.	11280 1X Liquid	11623 Powder mg/L
Component	mg/L	mg/L
INORGANIC SALTS:		
CaCl ₂ (anhyd.)	-	14.00
CaCl ₂ • 2H ₂ O	185.00	-
KCl	400.00	400.0
KH ₂ PO ₄ (anhyd.)	60.00	60.00
MgCl ₂ • 6H ₂ O	100.00	-
MgSO ₄ (anhyd.)	-	97.67
MgSO ₄ • 7H ₂ O	100.00	-
NaCl	8000.00	8000.00
NaHCO ₃	350.00	-
NaH ₂ PO ₄ (anhyd.)	48.00	47.80
OTHER COMPONENTS:		
D-Glucose	1000.00	1000.00
Lactalbumin Hydrolysate	6500.00	5000.00
Phenol Red	10.00	10.00